6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring arsenic, its metabolites, and other biomarkers of exposure and effect to arsenic. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Atomic absorption spectrophotometry (AAS) is the most common analytical procedure for measuring arsenic in biological materials (Curatola et al. 1978; Foa et al. 1984; Johnson and Farmer 1989; Mushak et al. 1977; Norin and Vahter 1981; Sotera et al. 1988). In AAS analysis, the sample is heated in a flame or in a graphite furnace until the element atomizes. The ground-state atomic vapor absorbs monochromatic radiation from a source and a photoelectric detector measures the intensity of transmitted radiation (APHA 1989b). Inductively-coupled plasma atomic emission spectrometry (ICP-AES) and ICP-mass spectrometry (ICP-MS) are increasingly common techniques for the analysis of arsenic; both methods can generally provide lower detection limits than absorbance detection methods.

Samples may be prepared for AAS in a variety of ways. Most often, the gaseous hydride procedure is employed (Curatola et al. 1978; Foa et al. 1984; Johnson and Farmer 1989; Norin and Vahter 1981). In this procedure, arsenic in the sample is reduced to arsine (AsH_3), a gas which is then trapped and introduced into the flame. This approach measures total inorganic arsenic, but may not detect all organic forms unless preceded by a digestion step. Digestion or wet-ashing with nitric, sulfuric and/or perchloric acids degrades the organic arsenic species to inorganic arsenic so that recovery of total arsenic from biological materials can be achieved (Maher 1989; Mushak et al. 1977; Versieck et al. 1983). For accurate results, it is important to check the completeness of the oxidation, however, this is seldom done (WHO 1981).

The arsenic concentration in biological fluids and tissues may also be determined by neutron activation analysis (NAA) (Landsberger and Simsons 1987; Versieck et al. 1983). In this approach, the sample is irradiated with a source of neutrons which converts a portion of the arsenic atoms to radioactive isotopes which can be quantified after separation from radioisotopes of other chemicals. Neutron activation has limited use because of the limited number of nuclear reactors in the United States providing this service and the need to dispose of radioactive waste. X-ray fluorescence is also capable of measuring arsenic in biological materials (Bloch and Shapiro 1986; Clyne et al. 1989; Nielson and Sanders 1983) and environmental samples (see Section 6.2). This method has the advantage that no sample digestion or separation steps are required. Hydride generation combined with atomic fluorescence spectroscopy (HG/AFS) is a relatively new technique that provides freedom from interference offered by hydride generation with sensitivity better than to 20 parts per trillion and linearity up to 10 ppm (PSA 2000).

Speciation of arsenic (i.e., analysis of organo-arsenicals or different inorganic species, rather than total arsenic) is usually accomplished by employing separation procedures prior to introduction of the sample material into a detection system. Various types of chromatography or chelation-extraction techniques are most commonly used in combination with AAS, ICP-AES, or ICP-MS detection methods (Dix et al. 1987; Foa et al. 1984; Johnson and Farmer 1989; Mushak et al. 1977; Norin et al. 1987; Thomas and Sniatecki 1995). In one method, HPLC is combined with HG/AFS to quantify As(III), dimethylarsinic acid (DMA), momomethyl arsonic acid (MMA), and As(V) (PSA 2000). Another approach involves selective reduction of arsenate and arsenite (permitting quantification of individual inorganic arsenic species), and selective distillation of methyl arsines to quantify MMA and DMA (Andreae 1977; Braman et al. 1977; Crecelius 1978). Most methods for measuring arsenic in biological samples, are unable to measure arsenobetaine with any accuracy because it does not form a hydride and it gives a different response from inorganic arsenic in electrothermal AAS. Ebdon et al. (1999) successfully employed using high performance liquid chromatography (HPLC) coupled with ICP-MS to determine arsenic speciation in blood plasma which was entirely ansenobetaine. Since marine foods are largely organic arsenic while inorganic arsenic compounds, being the most toxic forms of arsenic and therefore of highest concern, Øygard et al. (1999) developed a simple method to determine inorganic arsenic in biological samples. Their method, that involves initially distilling inorganic arsenic from the sample as $AsCl_3$ using HCl, avoids separating and quantifying all the different arsenic species which is both costly and time-consuming.

Table 6-1 summarizes a variety of methods for measuring total arsenic and individual arsenic species in biological materials. None of these methods have been standardized by EPA or other federal agencies.

Table 6-1. Analytical Methods for Determining Arsenic in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for total arsenic:					
Blood	Digestion with nitric acid and hydrogen peroxide; dry ash with magnesium oxide/magnesium nitrate; reduction with sodium borohydride	HGAAS	0.5 µg/L	95–102	Foa et al. 1984
Blood, hair	Wet ash with nitric/perchloric acids; reduction with sodium borohydride	HGAAS	0.1 µg/L[a]	95–105	Valentine et al. 1979
Serum	Irradiation; digestion with nitric/perchloric/sulfuric acids; extraction with toluene	NAA	0.088 ng/mL[a]	94–98	Versieck et al. 1983
Urine	Irradiate epithermally	NAA	40–100 ng/g	93–109	Landsberger and Simsons 1987
Urine	Digestion with nitric and perchloric acid; reduction with tin chloride; generation arsine by addition of zinc; reaction with SDDC	Colorimetric photometry	0.5 µg/sample	90–110	Pinto et al. 1976
Urine	Pre-treatment with L-cysteine; reduction with potassium iodide/ascorbic acid	Flow injection HGAAS	0.1 µg/L	95–100	Guo et al. 1997
Urine	Drying sample; irradiation with x-rays	XRF	0.2 µg/L[a]	92–108	Clyne et al. 1989
Hair	Wet ashing with nitric/sulfuric acids and hydrogen peroxide; reduction to arsine with sodium borohydride	HGAAS	0.06 µg/g	93	Curatola et al. 1978
Soft tissue	Digestion with nitric/sulfuric acids; complexation with DDDC in potassium iodide; extraction with chloroform	GFAAS	0.2 ppm	79.8	Mushak et al. 1977

Table 6-1. Analytical Methods for Determining Arsenic in Biological Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Nails	Wet ashing with nitric/sulfuric acids and hydrogen peroxide; reduction to arsine with sodium borohydride	HGAAS	1.5 µg/g	No data	Agahian et al. 1990
Methods for arsenic speciation:					
Urine	Separation of As^{+3}, As^{+5}, MMA, and DMA on anion/cation exchange resin column; reduction to respective arsines with sodium borohydride	IEC/HGAAS	0.5 µg/L	93–106	Johnson and Farmer 1989
Urine	Reduction of As^{+3}, As^{+5}, MMA, and DMA to arsines with sodium borohydride	HGAAS	0.08 µg/L	97–104	Norin and Vahter 1981
Urine	Reduction of As^{+3}, As^{+5}, MMA, and DMA to arsines; collection in cold trap; selective distillation by slow warming	Atomic emission (direct-current plasma)	#1 ng for all four species	No data	Braman et al. 1977
Urine	Extraction with chloroform/methanol; column separation with chloroform/ methanol; elution on cation exchange column with ammonium hydroxide	HGAAS/TLC/ HRMS	0.34 mg/ sample[a]	No data	Tam et al. 1982
Blood/tissue	Acidification with hydrochloric acid; complexation with TGM; extraction into cyclohexane; separation on capillary column	GLC/ECD	0.1 mg/mL	No data	Dix et al. 1987
Blood plasma	Separation by HPLC	HPLC/ICP-MS	2.5 ng As/mL	~100	Ebdon et al. 1999
Urine	Separation by anion exchange chromatography; detection by direct coupling of column to ICP-MS	IEC/ICP-MS	<0.45 µg/L for all species	No data	Inoue et al. 1994

Table 6-1. Analytical Methods for Determining Arsenic in Biological Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Marine biota	Extraction with methanol-water; removal of fats by liquid-liquid extraction or solid-phase cartridge	HPLC/ICP-MS	6–25 ng/mL	94.6 (fish muscle CRM)	Sniatecki 1994
Marine biota	Separation by anion exchange coupled with HPLC; on-line microwave oxidation	HPLC/HGAAS	0.3–0.9 ng	95–110 (recovery of spike in fish tissue)	López-Gonzálvez et al. 1994
Biological samples - Inorganic arsenic	Distill inorganic arsenic as $AsCl_3$ using HCl after pre-reduction of As(V) with KI/HCl	Flow-injection HGAAS	0.045 mg/kg (dry matter)	No data	Øygard et al. 1999

[a] Lowest reported concentration

CRM = certified reference material; DDDC = diethylammonium diethyldithiocarbamate; DMA = dimethylarsinate; ECD = electron capture detector; GFAAS = graphite furnace atomic absorption spectrometry; GLC = gas-liquid chromatography; HGAAS = hydride generation atomic absorption spectrometry; HRMS = high resolution mass spectrometry; ICP-MS = inductively-coupled plasma mass spectrometry; IEC = ion exchange chromatography; HPLC = high-performance liquid chromatography; MMA = monomethylarsonate; NAA = neutron activation analysis; SDDC = silver diethyldithiocarbamate; TGM = thioglycolic acid methylester; TLC = thin layer chromatography; XRF = x-ray fluorescence

Detection limits in blood and urine are about 0.1–1 ppb for most techniques; limits for hair and tissues are usually somewhat higher.

6.2 ENVIRONMENTAL SAMPLES

Arsenic in environmental samples is also measured most often by AAS techniques, with samples prepared by digestion with nitric, sulfuric and/or perchloric acids (Dabeka and Lacroix 1987; EPA 1983b, 1994a, 1994b; Hershey et al. 1988). Other methods employed include a spectrophotometric technique in which a soluble red complex of arsine and silver diethyldithiocarbamate (SDDC) is formed (APHA 1977; EPA 1983c), ICP-AES (EPA 1982b, 1996a), graphite furnace AAS (EPA 1994b), ICP-MS (EPA 1998j), and X-ray fluorescence (Khan et al. 1989; Nielson and Sanders 1983).

Since arsenic in air is usually associated with particulate matter, standard methods involve collection of air samples on glass fiber or membrane filters, acid extraction of the filters, arsine generation and analysis by SDDC spectrophotometry or AAS (APHA 1977; NIOSH 1984).

Methods standardized by the EPA for measuring total arsenic in water and wastewater, solid wastes, soil and sediments include: ICP-MS (EPA 1998j, 1994a, 1991), ICP-AES (EPA 1996d), graphite furnace AAS (EPA 1994b), quartz furnace hydride generation AAS (EPA 1996h) and an electrochemical method using anodic stripping voltammetry (ASV) (EPA 1996e). A modification using cryogenic GC to EPA Method 1632 (HG/AAS) allows the technique to be adopted for the species As(III), As(V), MMA, and DMA to the 0.003 ppb level (1996f). Similar methods are recommended by APHA for water using AAS/hydride generation (APHA 1989c), AAS/graphite furnace technique (APHA 1989b), ICP (APHA 1989d), or SDDC spectrophotometry (APHA 1989a). The AAS/hydride generation method is generally resistant to matrix and chemical interferences (APHA 1989a). Techniques to compensate for these interferences have been described by EPA (1982b).

Analysis for arsenic in foods is also most frequently accomplished by AAS techniques (Arenas et al. 1988; Dabeka and Lacroix 1987; Hershey et al. 1988; Tam and Lacroix 1982). Hydride generation is the sample preparation method most often employed (Arenas et al. 1988; Hershey et al. 1988), but interferences must be evaluated and minimized.

Speciation of inorganic arsenic in environmental samples is usually accomplished by chromatographic separation, chelation-extraction or elution of As^{+3} and then reduction of As^{+5} with subsequent similar

treatment (Butler 1988; López-Gonzálvez et al. 1994; Mok et al. 1988; Rabano et al. 1989). Methods are also available for quantifying organic arsenicals in environmental media, including arsenobetaine in fish (Beauchemin et al. 1988; Cannon et al. 1983) and other organic forms of arsenic in water, soil, and foods using hyphenated methods of separation and detection (HPLC/ICP-MS, HPLC/HGAAS, IC/ICP-MS) (Andreae 1977; Braman et al. 1977; Comber and Howard 1989; Crecelius 1978; Heitkemper et al. 1994; López-Gonzálvez et al. 1994; Odanaka et al. 1983; Teräsahde et al. 1996).

A summary of selected methods for analysis of total arsenic and individual inorganic and organic arsenic species in environmental samples is presented in Table 6-2.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of arsenic is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of arsenic.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The most useful biomarkers of exposure to arsenic are levels of arsenic in urine, hair, or nails. Existing methods are sufficiently sensitive to measure background levels of arsenic in these tissues for average persons, and to detect increases as a result of above-average exposure (Agahian et al. 1990; Curatola et al. 1978; Clyne et al. 1989; Foa et al. 1984; Gebel et al. 1998b; Landsberger and Simsons 1987; Mushak et al. 1977; Pinto et al. 1976; Valentine et al. 1979; Versieck et al. 1983). The precision and accuracy of these methods are documented. Methods are also available that can distinguish nontoxic forms of arsenic (arsenobetaine)

Table 6-2. Analytical Methods for Determining Arsenic in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for total arsenic:					
Air (particulates)	Collection on cellulose ester membrane filter; digestion with nitric acid, sulfuric acid, and perchloric acid	NIOSH Method 7900; HGAAS	0.02/sample	No data	NIOSH 1994
Air (particulate arsenic and arsenic trioxide vapor)	Collection on Na_2CO_3-impregnated cellulose ester membrane filter and H_2O_2	NIOSH Method 7901; GFAAS	0.06/sample	No data	NIOSH 1994
Air	Collection on cellulose ester membrane filter; digestion with nitric acid, sulfuric acid, and perchloric acid	NIOSH Method 7300; ICP-AES	0.001 µg/m^3	No data	NIOSH 1994
Water/waste water	Digestion with nitric acid	EPA Methods 200.7 and 6010B; ICP-AES	35 µg/L	86–105	EMMI 1997; EPA 1982c, 1996d
Water/soil/solid waste	Digestion with nitric acid and hydrogen peroxide	EPA Methods 206.2 and 7060A; GFAAS with $Ni(NO_3)_2$ modifier	1 µg/L	85–106	EPA 1983b, 1994b
Water/waste water/ solid waste	Digestion with nitric acid	EPA Methods 200.8, 6020 and 6020A; ICP-MS	0.4 µg/L	97–114	EMMI 1997; EPA 1991, 1994a, 1998j
Water/soil/solid waste	Digestion with nitric/sulfuric acid; reduction to As^{+3} with tin chloride; reduction to arsine with zinc in acid solution	EPA Methods 206.3 and 7061; HGAAS	2 µg/L	85–94	EPA 1983c, 1986d

Table 6-2. Analytical Methods for Determining Arsenic in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Reduction to arsine in acid solution; reaction with SDDC	EPA Method 206.4; SDDC colorimetric spectrophotometry at 510 nm	10 µg/L	100	EPA 1983d
Water	Digestion with 6M HCl; reduction to arsine wth sodium borohydride; cold trap and desorption into quartz furnace	EPA Method 1632; HGAAS	2 ng/L	No data	EMMI 1997; EPA 1996h
Food	Digestion with nitric acid; dry ashing with magnesium oxide; reduction with ascorbic acid; precipitation with APDC in presence of nickel carrier	GFAAS	10 ng	86–107	Dabeka and Lacroix 1987
Food	Digestion with nitric/sulfuric/perchloric acids; reduction to trivalent arsenic with potassium iodide; reduction to arsine with sodium borohydride	HGAAS	0.1 µg/g	98–110	Hershey et al. 1988
Soil, rock, coal	Preparation of pellet	XRF (backscatter)	4 mg/kg	SRM recoveries: 110±4 in soil; 100±1in rock; 97±18 in coal	Nielson and Sanders 1983
Methods for species of arsenic:					
Air (particulate organoarsenals)	Collection on PTFE filter	NIOSH Method 5022; ion chromatography/ HGAAS	0.2 µg As/sample	No data	NIOSH 1994

Table 6-2. Analytical Methods for Determining Arsenic in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air particulates (As^{+3} and As^{+5} only)	Collection on PFTE filter in high volume dichotomous virtual impactor; desorption with ethanolic hydrochloric acid; selective reduction of As^{+3} to arsine with zinc in acid and reduction of As^{+5} to arsine with sodium tetrahydrodiborate	HGAAS	1 ng/m^3	95±7 (As^{+3}) 100±8 (As^{+5}) on spiked materials	Rabano et al. 1989
Water	Selective elution of As^{+3} with orthophosphoric acid; elution and conversion of As^{+5} to As^{+3} with sulfur dioxide	IEC/amperometric detector (detects As^{+3} only)	0.9 µg/L	95% of converted As^{+5} recovered	Butler 1988
Water/soil	Selective complexation of As^{+5} with ammonium molybdate; extraction with isoamyl alcohol to separate from As^{+3}	Colorimetric spectrometry at 712 nm	No data	No data	Brown and Button 1979
Water	Selective extraction extraction of As^{+3} with APDC into chloroform; back extraction with nitric acid; reduction of As^{+5} to As^{+3} with thiosulfate and extract	NAA	0.01 ppb	No data	Braman et al. 1977
Food (arsenobetaine in fish)	Extraction of arsenobetaine with methanol/chloroform; digestion with nitric acid/magnesium nitrate for remainder of As species	HPLC/ICP-MS	0.3 ng as arsenobetaine	101±4 recovery of arsenobetaine	Beauchemin et al. 1988
Water/waste water/soil (inorganic species)	Acidification or digestion with hydrochlroic acid	EPA Method 7063; ASV	0.1 µg/L	96–102	EPA 1996e

Table 6-2. Analytical Methods for Determining Arsenic in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Cryogenic GC, Digestion with 6M HCl; reduction to arsine wth sodium borohydride; cold trap and desorption into quartz furnace	EPA Method 1632 appendix; HGAAS	3 ng/L	No data	EPA 1996h
Water	Reduction to arsines; cold trap and selectively warm to separate arsine species	AAS	2 ng/L	91–109	Andreae 1977
Water	Reduction of MMA, DMA and inorganic As (control pH to select As^{+3} or As^{+5}) to arsines with sodium tetrahydroborate; cold trap and selectively warm to separate arsine species	HGAAS	0.019–0.061 ng	No data	Comber and Howard 1989
Water/soil	Extraction with sodium bicarbonate; reduction of inorganic arsenic, MMA and DMA to hydrides with sodium borohydride; cold trap arsines in *n*-heptane	HG-HCT/GC-MID	0.2–0.4 µg/L	97–102	Odanaka et al. 1983

AAS = atomic absorption spectrophotometry; APDC = ammonium pyrrolidine dithiocarbamate; ASV = anodic stripping voltammetry; DMA = dimethylarsinate; EPA = Environmental Protection Agency; GC-MID = gas chromatography-multiple ion detection; GFAAS = graphite furnace atomic absorption spectrometry; HGAAS=hydride generation-atomic absorption spectroscopy; HG-HCT = hydride generation-heptane cold trap; HPLC = high perpformance liquid chromatography; ICP-AES = inductively coupled plasma-atomic emission spectrometry; ICP-MS = inductively coupled plasma-mass spectrometry; IEC = ion exchange chromatography; MMA = monomethylarsonate; NAA = neutron activation analysis; NIOSH = National Institute of Occupational Safety and Health; PTFE = polytetrafluoroethylene; SDDC = silver diethyldithiocarbamate; SRM = standard reference material; XRF = X-ray fluorescence

from inorganic and organic derivatives that are of health concern (Braman et al. 1977; Dix et al. 1987; Johnson and Farmer 1989; Norin and Vahter 1981; Tam et al. 1982). Further efforts to improve accuracy, reduce interferences and detect multiple species using a single analysis would be valuable.

Arsenic is believed to act by inhibition of numerous cell enzymes and/or by interfering with phosphate metabolism, and effects on several enzyme systems have been characterized in animals and *in vitro*. However, these effects are not specific to arsenic, and most can only be measured in tissue extracts. Efforts to identify an arsenic-specific enzymic or metabolic effect would be valuable, particularly if the effect could be measured using non-invasive techniques, and if the effect were specifically linked to the dermal, neurological, or hematological injuries that are characteristic of arsenic toxicity.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Arsenic is ubiquitous in the environment. It is found in air, water, soil, sediments, and food in several inorganic and organic forms. Analytical methods exist for the analysis of arsenic species in all of these environmental media, and these methods have the sensitivity to measure background levels and to detect elevated concentrations due to emissions from sources such as smelters, chemical plants, or hazardous waste sites (APHA 1977, 1989c; EPA 1982b, 1983a, 1983b, 1983c, 1991, 1994b, 1996a, 1996f). However, further research to reduce chemical and matrix interferences may improve the speed and accuracy of the analyses.

6.3.2 Ongoing Studies

Methods to reduce detection limits, decrease interferences by other elements, and investigate applications of different separation methods for arsenic species are being pursued (FEDRIP 1998).